Mississippi Canyon 252 Oil Spill

NRDA Sampling Plan

Assessment of Impacts from the Deepwater Horizon Oil Spill on Red Crabs

Deepwater Benthic Communities Technical Working Group

Approval of this work plan is for the purposes of obtaining data for the Natural Resource Damage Assessment. Each party reserves the right to produce its own independent interpretation and analysis of any data collected pursuant to this work plan.

The Trustees have developed a preliminary conceptual model of the Deepwater Horizon (DWH) release, potential pathways and routes of exposure, and potential receptors. This preliminary model has informed the Trustees' decision to pursue the studies outlined in the work plan. By signing this work plan and agreeing to fund the work outlined, BP is not endorsing the model articulated in the work plan.

This plan will be implemented consistent with existing Trustee regulations and policies. All applicable state and Federal permits must be obtained prior to conducting work.

The cruise described in this plan was conducted prior to its finalization and signature.

APPROVED:

Robert W Ricker — Feb 13, 2013

Department of Commerce Trustee Representative: Date

Joyce Mikey — Feb 14, 2013

BP Representative: Date

Louisiana Trustee Representative: 3/8/13 Date

Assessment of Impacts from the Deepwater Horizon Oil Spill on Red Crabs
Deepwater Benthic Communities Technical Working Group

January 9, 2013

Cruise Vessel: NOAA R/V Pisces
Cruise Dates: July 27 – Aug 7, 2011; Aug 8 – Aug 17, 2011

1.0 Background and Objectives:

Macrofaunal benthic communities may have been adversely affected by exposure to DWH oil, dispersants, or other direct and indirect effects of the oil spill. Deep water crabs of the family Geryonidae are widely distributed throughout the world's oceans (Manning and Holthuis 1989). Crabs of the genus Chaceon (formerly Geryon) are fished for human consumption along both sides of the Atlantic Ocean including the coastal areas of southwest Africa, the eastern United States, and Bermuda (Beyers and Wilke 1980, Lux et al. 1982, Manning and Holthuis 1986, Erdman and Blake 1988). Two species are known to occur in the Gulf of Mexico (GOM), and studies in the northeastern and north central GOM have demonstrated a potential for limited commercial fisheries harvest (Lockhart et al. 1990, Waller et al. 1995). One of the two species, and the species of focus for this work plan: the red crab, *Chaceon quinquedens*, is distributed in deep waters of the northern, eastern, and western GOM (Lockhart et al. 1990, Waller et al. 1995, Pequegnat 1975) and is most abundant off the mouth of the Mississippi River. Weinberg et al. (2003) suggested that genetic differences between north Atlantic and GOM stocks of *C. quinquedens* were great enough to consider them to be two separate biological populations and stocks. Diehl and Biesiot (1994) noted that there was a high degree of genetic variability in red crabs that could mask evidence of a trend toward genetic separation.

A limited-entry fishery exists for red crabs in the northwest Atlantic with four to five vessels harvesting approximately 2000 tons annually (Wahle et al. 2008). A fishery management plan for this stock was initiated in 2002 and all red crabs in the US waters outside the Gulf of Mexico are currently managed as one unit (Chute et al. 2008). Using various estimates for effective fishing area of a trap, Waller et al. (1995) derived population estimates of the number of fishable crabs on trapping grounds of the north-central GOM that ranged from 3.7 to 10.7 million individuals.

Studies of geryonids in the GOM include: 1) Lindberg and Lockhart (1993), Lockhart et al. (1990), and Waller et al. (1995) - geographic and bathymetric distribution, abundance, and size of geryonid crabs in the northern and eastern GOM, 2) Henry et al. (1990 a,b) - physiology of the two species, 3) Beisiot and Perry (1995) - biochemical composition of *C. quinquedens*, 4) Erdmann et al. (1991) – comparative reproduction of the two species, 5) Perry et al. (1995) - trap bycatch, 6) Stuck et al. (1992) – larval development of *C. fenneri*, 7) Trigg et al. (1997) - size and weight relationships for the two species, and 8) CSA 2006 – metals and hydrocarbon concentrations in red crabs at MC 292 and the Garden Banks (GB602). There are numerous studies of *C. quinquedens* from Atlantic waters that contain ecological and harvesting data, some of which may be relevant to the GOM population.

Red crabs generally inhabit soft bottom areas and are an integral part of the benthic community. As bottom dwelling organisms, red crabs continuously filter fine sediment particles that are present in the bottom mud and that section of the water column immediately adjacent to the sediment. Hastie (1995) suggested that red crabs are major bioturbators of surface sediments through their foraging and feeding activities and are important biotic modifiers of the sea floor. Lindberg and Lockhart (1993) reported that red crabs in the eastern GOM were buried just under the sediment and emerged when approached by an ROV. They described their habitat as bioturbated lime-mud with large pits and mounds.

Observations on migrations and movements made by Ganz and Herrmann (1975) and Lux et al. (1982) off southern New England suggest that adult movement up and down the slope (ranging up to 500 m) may be related to spawning and while there was some lateral movement along the slope up to almost 100 km, tagging studies found that most returns were within 20 km of the release site. Lockhart et al. (1990) also proposed the possibility of a seasonal downslope movement to explain the differences in catch of red crabs during some seasons.[1] Reproduction of *C. quinquedens* in the GOM was described by Erdmann et al. (1991). Red crabs exhibit an annual reproductive cycle with oviposition beginning in May. Erdmann et al. (1991) noted that although the species reproduces annually, individuals may reproduce biennially as evidenced by the long brooding period, low numbers of molting females, and the high incidence of non-ovigerous females collected during the brooding season. Eggs are brooded for 9 months with hatching in February/March. Hatching in the spring coincides with warming of surface waters and increased productivity. Eggs are large, ranging from 630-850 µm, and their size provides nutritional flexibility to the larvae that migrate from deep waters to the surface for larval development.

This plan describes assessment activities targeting red crabs in the vicinity of the DWH well head. Specifically, the Trustees and BP will set and retrieve baited crab traps to collect red crabs for analysis. The objectives of this work plan are the following:

1) To collect and document potential exposure of red crabs to spill-related contaminants, including petroleum hydrocarbons, metals, and dispersant or dispersant by-products;
2) To collect tissue samples to document potential reproductive and histological effects of exposure to petroleum hydrocarbons, metals, and dispersant or dispersant by-product; and,
3) To collect information on catch per unit effort (CPUE) at selected study locations, including near field, historic, and seeps locations.

[1] Lockhart et al. (1990), Lindberg and Lockhart (1993), and Waller et al. (1994) provide reference data on population structure and bathymetric/geographic distribution and abundance. Each of these studies has data for the August/September time period, and thus may be comparable to data collected in the proposed study. Long-distance movements of red crabs in the GOM are associated with mating in the spring and a downward shift in the population occurs as mated pairs move downslope (Lockhart et al. 1990). Movement during other seasons is restricted and associated with short distance migrations associated with foraging.

2.0 Methods and Approach:

2.1. Objective 1: To collect and document exposure of red crabs to spill-related contaminants, including petroleum hydrocarbons, metals, and dispersant or dispersant by-products.

Red crabs will be collected using baited traps at 12 locations in the northern GOM to assess exposure to hydrocarbons, metals and dispersants.[2,3] Seven baited Fathoms Plus traps will be deployed by line at each location, for an approximate duration of 18 hours; after which they will be retrieved. Trapping will be conducted from the National Oceanic and Atmospheric Administration (NOAA) R/V *Pisces*. Sample sites will include historic Gulf Coast Research Lab (GCRL) trapping sites (Areas 1, 2, 4, and 6; "historic" locations) described in Lockhart et al. (1990) and Waller et al. (1994), three locations in close proximity to the Macondo well head and one location approximately 11 km from the well head with potential oiling from the DWH incident ("near field" locations), and four locations at naturally occurring oil seep sites, some of which may have experienced potential oiling from the DWH incident ("seeps" locations; Table 1, Figure 1). The target sample size is 40 crabs from each site.[4] However, only two trapping lines will be set per site, regardless of catch. A sampling flow-chart is provided in Figure 2. All samples generated as part of the sampling will be tracked at the individual crab level.

[2] Metals that will be analyzed will include: Al, Sb, As, Ba, Be, Cd, Ca, Cr, Co, Cu, Fe, Pb, Mg, Mn, Hg, Ni, K, Se, Ag, Na, Tl, V, Zn, and Sr.

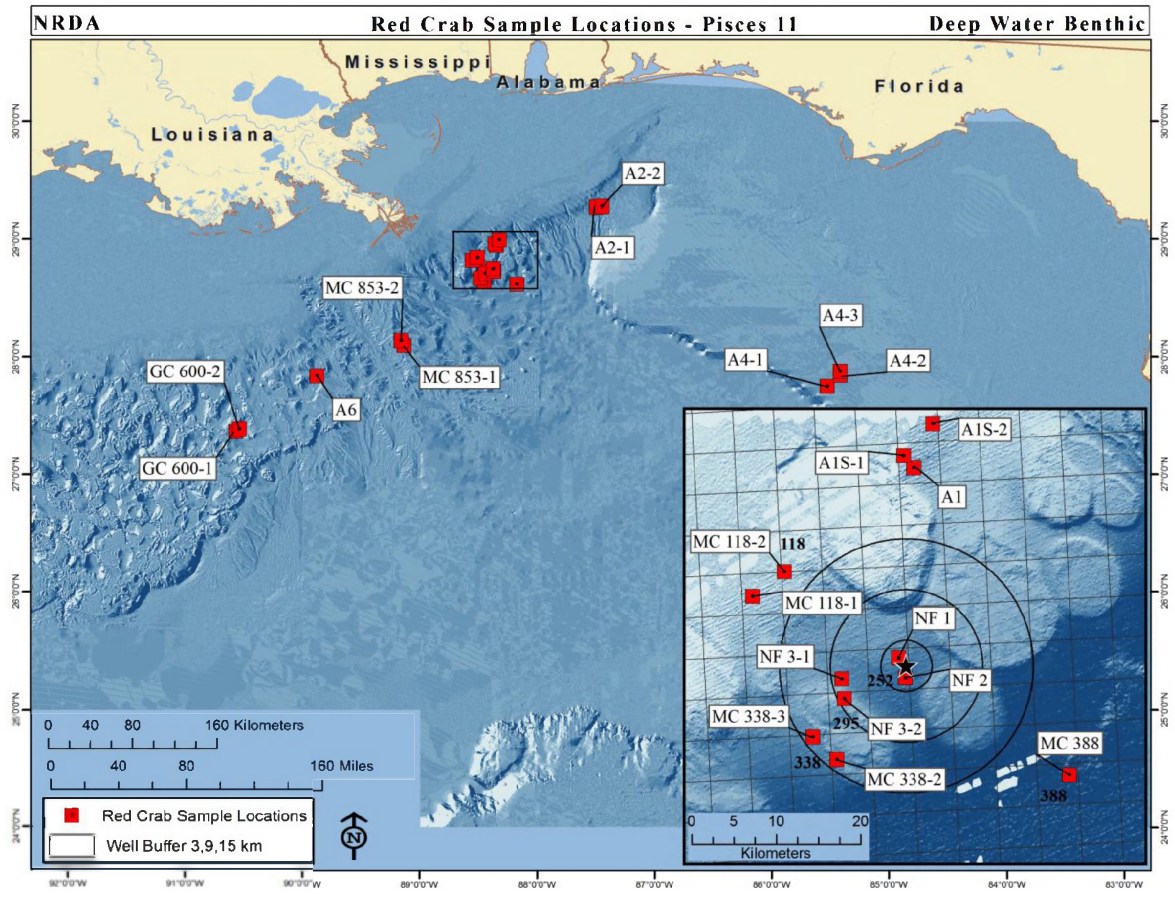
[3] Limited pre-release baseline metals and PAH concentrations in red crab tissues sampled in the vicinity of offshore drilling rigs are available in CSA (2006).

[4] If more than 40 crabs are collected at a given site, excess crabs will be photographed, standard metrics recorded, wrapped in aluminum foil, and frozen for archive. All by-catch will be identified, if possible, and treated in this same manner.

Table 1. Actual Crab Sampling Location by Trap Line Set

Site ID	*Date Deployed*	*Latitude (Degrees North)*	*Longitude (Degrees West)*
Area 1A Shallow A	8/14/2011	28.994079	88.32239217
Area 1A Shallow B	8/15/2011	28.97797333	88.35870867
Area 1 Deep	8/14/2011	28.94817467	88.34695317
Area 2A	8/9/2011	29.27712767	87.44381217
Area 2B	8/9/2011	29.26998567	87.49329533
Area 4A	7/28/2011	27.8415825	85.41578583
Area 4B	7/28/2011	27.8742315	85.41605433
Area 4C	7/29/2011	27.74831533	85.52738483
Area 6A	8/2/2011	27.80253383	89.8651175
Area 6B	8/2/2011	27.8353605	89.871941
Near Field 1	8/4/2011	28.74663517	88.37424817
Near Field 2	8/5/2011	28.7257195	88.36675517
Near Field 3A	8/10/2011	28.70592567	88.44177867
Near Field 3B	8/10/2011	28.71142367	88.491149
MC338A	8/11/2011	28.66636933	88.48149817
MC338B	8/11/2011	28.64154883	88.45367
MC853A	8/3/2011	28.13837317	89.15238383
MC853B	8/3/2011	28.09426067	89.12778
MC118A	8/15/2011	28.8430045	88.51413433
MC118B	8/15/2011	28.823882	88.5482195
MC388A	8/12/2011	28.61545	88.1741725
MC388B	8/13/2011	28.617601	88.13233017
GC600A	7/31/2011	27.3646575	90.56392983
GC600B	7/31/2011	27.384278	90.53343783

Figure 1. Actual Sampling Locations



Upon arrival at the surface, collected crabs will be removed from traps and will be immediately placed in a refrigerated seawater system (Frigid Units, Inc.) maintained at 5 °C. Crabs will be maintained alive for a maximum of 24 hours in refrigerated seawater until dissected onboard. Crabs with any visual contamination will be stored in buckets filled with chilled seawater. In cases where the number of crabs captured exceeds tank capacity, the excess crabs will be maintained in refrigerated seawater in five-gallon buckets and these crabs will be dissected first. Further, seawater will be changed and the refrigerated seawater systems cleaned between each sampling location. To clean the seawater system, the tanks, tubing, and airstones will be rinsed with a high-pressure freshwater hose. Seawater will be changed between each sampling location[5]; with seawater collected at the location and time where and when traps are deployed and allowed to cool to 5 °C while the traps are in the water. The seawater systems will be located inside the vessel in the wet laboratory and will be kept covered to reduce exposure to ship exhaust. Salinity will be adjusted to 35 ppt using instant ocean sea salts. In addition, three water samples from any refrigerated seawater systems being used to store crabs will be taken for analysis of hydrocarbons, metals, and dispersants, respectively. All crab traps and lines will be

[5] As a precaution against contamination from natural slicks or vessel exhaust, only subsurface seawater will be used (intake 19 feet below surface).

cleaned with soap and freshwater or site water in a designated decontamination area of the vessel between sampling locations, and stored in the laboratory when not in use.

Prior to dissection, the dorsal and ventral side of each crab will be photographed along with its identification number.[6] Routine biological data to be recorded for each crab will include: carapace width and length (mm), weight (gm), sex, molt stage, missing appendages, egg color (ovigerous females) and general appearance. Molt stage will be determined by external characteristics (hardness of shell and color).

Crabs designated for dissection will be dissected on a glass cutting board using ceramic or Teflon instruments. Dissection equipment will be decontaminated between each crab. Once the carapace is removed, the internal organs will be photographed to determine gross ovarian condition (see Objective 2 below). The following tissues will be excised: gills, hepatopancreas, gonads, and muscle[7]. If ovigerous females are collected, a sample of the egg mass will be taken. Sub-samples will be taken for histological analysis (see Objective 2 below). Tissue samples for contaminants exposure analysis will be weighed (if sea conditions permit) prior to being placed in a labeled, trace-clean jar and frozen onboard the vessel at -20°C. Target masses from each crab from each tissue type for analyses are the following: 30 g for hydrocarbon analysis, 5 g for metals analysis, and 10 g for dispersant analysis[8]. If insufficient tissue is collected from the individual crab, then PAH analyses will take precedence.

Between each station and each set, gear will be cleaned with laboratory soap and then rinsed with fresh water or sea water from the site. When the gear is not being used it will be stored in the laboratory.

Between each crab, the laboratory area and all the tools and equipment used to dissect crabs will be cleaned with laboratory soap, rinsed with isopropyl alcohol, and then rinsed with deionized distilled water. In order to avoid cross contamination, individual tools will be marked for external/internal use and stored separately on the laboratory bench. If contamination is suspected or observed, tools will be re-cleaned or replaced with 'clean tools'.

Equipment blanks of the dissection work area and the refrigerated seawater system will be collected daily. Equipment blanks will be collected with the following procedures:

- The refrigerated seawater system and all dissecting tools, including the cutting board and instruments will be decontaminated with Alconox, isopropyl alcohol, and water.
- The tools and cutting board and equipment will be rinsed with laboratory-grade de-ionized water.

[6] Each crab collected will be individually identified based on the sampling location and trap.
[7] Gills will be laboratory analyzed to determine presence/absence of oil on these structures as oil on or in the gills may interfere with respiration and other physiological processes. Decapods store organic reserves from dietary sources in the hepatopancreas and this organ has been shown to accumulate metals and oil/oil-related contaminants.
[8] With the exception of egg samples, samples for hydrocarbon, metals, and dispersant analyses will be aliquoted on board. Egg samples designated for contaminants analysis will be sent in an individual sample jar, and will be alliquoted at Alpha Analytical Laboratory.

- The rinse water will be collected and stored in a sterile sample bottle and submitted to the laboratory for analysis of PAHs, total extractable hydrocarbons, dispersants and metals.

All tissue samples for contaminants analysis will be transported frozen to the NRDA/Trustee analytical chemistry contractors for extraction and analysis of contaminants using approved NRDA AQAP chemistry analysis and laboratory methods. Analyses will follow methods provided in the NOAA MC 252 Analytical Quality Assurance Plan (AQAP) version 3.0 (NOAA 2011).

Samples for hydrocarbon analysis will be sent frozen to Alpha Analytical Laboratories. To the extent that low-mass analytical techniques become available for use in the NRDA (e.g., TOF-DART), low-mass analyses may be used to assess hydrocarbon content. Low mass samples not suitable for traditional analysis will either be archived or composited at the laboratory for analysis. In the latter circumstance, the samples comprising the composited sample will be recorded. PAHs will be the preferred analysis and metals and/or dispersant analysis may not be performed if compositing is not possible and/or if there is insufficient sample size for analysis. The following measurements will be made: PAHs including individual parent and alkyl homologues as well as petroleum biomarker compounds as identified in Table 1.1a and 1.1e, respectively of the AQAP version 3.0. Egg samples sent to Alpha Analytical Laboratories will be enumerated using a "wet method" prior to analysis for contaminants to complement fecundity measures (see Objective 2 below). Specifically:

- The full egg sample will be thawed and weighed to the thousandth of a gram,
- Three subsets of eggs (approximately 250 eggs) will be counted and weighed, and
- The total number of eggs in the full sample will be calculated using the average egg-to-mass ratio of the egg sub-samples.

The full egg sample, including the sub-samples, will then be subdivided for subsequent chemical analyses.[9]

Tissue sub-samples will also be analyzed for metals (i.e., the Target Analyte List (TAL) and Strontium) that are common constituents of drill cuttings and drill muds (Kennicutt et al. 1996), as well as Corexit 9500/9527 dispersant indicators (e.g., dioctylsulfosuccinate sodium salt, Dipropylene Glycol n-Butyl Ether). Sub-samples for metals analysis will be transported frozen to Alpha Analytical Laboratory for extraction and analysis. Sub-samples for Corexit analysis will be transported frozen to Columbia Analytical Services for extraction and analysis.

2.2. Objective 2: To collect tissue samples to document potential for reproductive and histological effects of exposure to petroleum hydrocarbons, metals, and dispersant or dispersant by-product.

Sub-samples of each tissue type collected for exposure analysis will be collected for histological analysis. Tissue subsamples will be excised and preserved on board in modified Davidson's

[9] For the egg samples, the sub-sample for dispersant analysis will be refrozen and shipped to Columbia Analytical Services for extraction and analysis. Sub-samples for metals and hydrocarbons will be extracted and analyzed at Alpha Analytical Laboratory immediately after enumeration.

Fixative. Upon return to shore, with the exception of egg samples, tissues will be processed by personnel at the NOAA Northwest Fisheries Science Center (NWFSC) using standard histological procedures: dehydration, clearing and embedding (Yevich and Barszcz 1977). Sections will be cut and the resulting slides stained in hematoxylin and eosin. Histological analyses will examine histopathological alterations/lesions of infectious and noninfectious nature as well as normal morphological parameters. Histological slides will be read by certified histologists at NOAA NWFSC.

Reproductive Histology: Reproductive histology may be used to evaluate potential changes in the reproductive cycle and stages of ovarian development relative to historical Gulf data (Erdman et al. 1991) and the data of Haefner (1977) from the Atlantic Coast. Ovarian development is supported by recent food intake and stored organic reserves. To the extent that crabs are stressed and/or expending energy due to sub-lethal exposure to oil, oil-related contaminants, or response activities, chronic physiological responses manifested by changes in reproductive tissue may be quantified when compared to historic data.

As noted above, once the carapace is removed, the internal organs will be photographed to determine gross ovarian condition (immature, early, intermediate, advanced, mature, and redeveloping/spent) using the methods of Haefner (1977) and Erdman (1990). Then, ovarian tissues will be sampled from all collected female crabs.[10]

For ovigerous females, the egg mass will be removed from the pleopod and subsampled for reproductive assessment by personnel at NOAA AKFSC[11]. Presence and color of extruded eggs will be recorded. Subsampling will be conducted to assess staging, morphometry, and fecundity (Attachment 1). Fecundity will be determined for each individual using published methodologies (Hines 1988). Because only a sub-set of eggs will be sent to NOAA AKFSC for fecundity determinations (with some eggs being sent to Alpha Analytical Laboratories for PAH analyses) fecundity will be calculated by summing the numbers of eggs enumerated through enumeration at NOAA AKFSC and Alpha Analytical Laboratories.

[10] The ovary is H-shaped in form and sections will be taken from the left anterior portion of the H, the right lower portion of the H and from the connecting portion.

[11] Crabs collected for fecundity analysis are in the set of crabs used for tissue chemistry analyses.

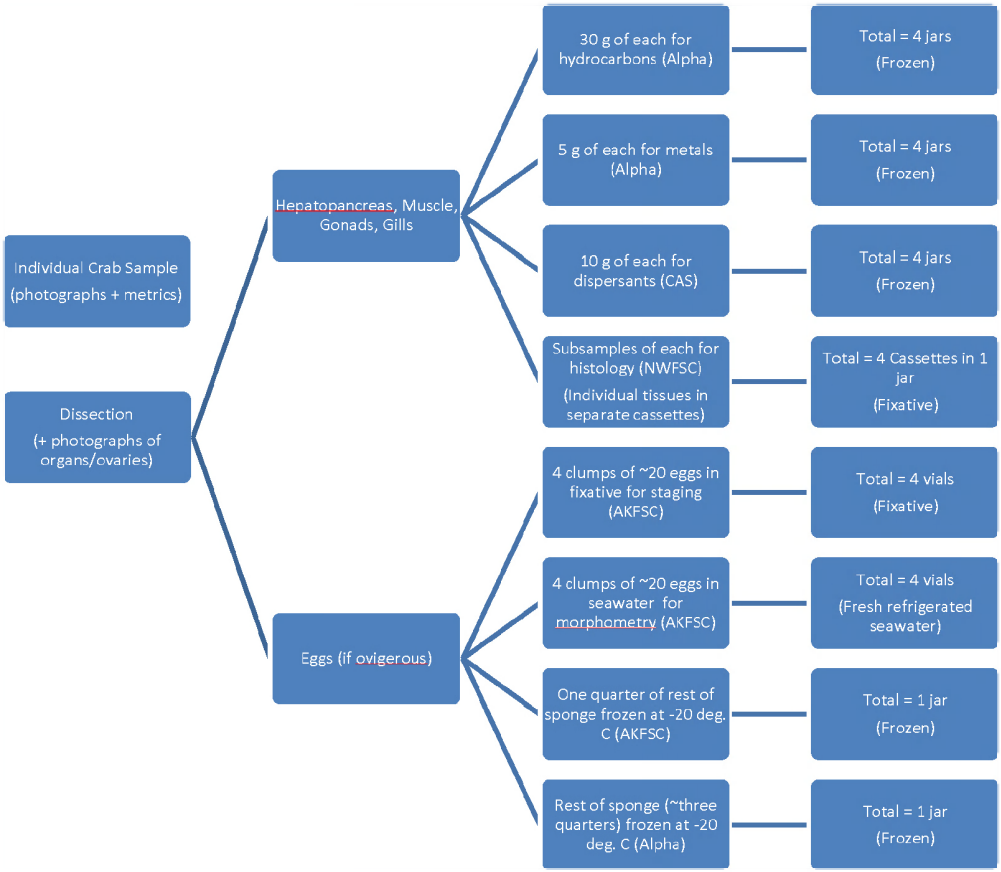


Figure 2: Sampling Flow-chart

2.3 Objective 3: To collect information on CPUE at selected study locations, including near field, historic, and seeps locations.

Total numbers of crabs collected will be counted. In addition, as noted above, standard crab metrics will be collected. Pre-spill data on CPUE, including historic collections in Area 1, are available from Lockhart et al. (1990), and Waller et al. (1995).

Selected by-catch will be wrapped in aluminum foil, labeled, and frozen whole.

3.0 Milestones and Deliverables:

- Cruise Report — Within two weeks of signature of the plan by BP. The cruise report will include the following sections:
    - Background and Objectives
    - Cruise Dates and Personnel
    - Approach and Methodologies
        - Station Locations
        - Water Quality

        - Gear Description and Deployment/Retrieval
        - Crab Maintenance Onboard
    - Biological Data
        - Dissection of Red Crabs
        - Dissection of Bait
    - Summary of samples for laboratory analyses
        - Sample ids, number of samples, collection locations and dates of samples for each analysis type (egg and tissue chemistry, tissue histology, reproduction, reproductive histology, population metrics)

4.0 Key Personnel:

Harriet Perry, University of Mississippi Gulf Coast Research Laboratory, Chief Scientist
Sean Sol, NOAA, NOAA NWFSC
Robert Foy, NOAA AKFSC
Mark Myers, NOAA NWFSC

5.0 Safety Plans:

A HASP binder containing all health and safety protocols is provided to each vessel. All well-established health and safety protocols will be followed and will be provided to the vessel in a dedicated binder. The NOAA *Pisces* is the vessel for the cruise. The ship's operational safety procedures will be followed at all times. Also, all activities will follow protocols of NOAA's Deepwater Horizon NRDA Field Safety Plan, latest version 1/28/2011 (NOAA 2011), which will be available on the vessel. MSDS hazardous materials sheets will be posted as well. Principal investigators may merge these safety plans with other applicable university or participating organization practices.

6.0 Data Sharing:

6.1 Digital and Shipboard Data

All data and imagery (including navigation, instrument data, field logs, photographs and documentation), acoustic, and other electronic data will be saved to an on-board computer, and all data shall be migrated to a dedicated external hard drive. The data will be controlled and managed by the NOAA NRDA data manager under project protocols, including Chain-of-Custody tracking of the external hard-drive. Upon return to port, the Data Manager shall deliver copies of all data on the external hard-drive, including copies of Chain-of-Custody forms, to the Trustees and Cardno ENTRIX on behalf of BP simultaneously by uploading all data to the NOAANRDA.org website.

Under the direction of the Chief Scientist, a NOAA Data Manager on board each vessel will email a daily report to a designated list of recipients that includes the Trustee representatives and BP/Cardno ENTRIX.

6.2 Laboratory Data

Tissue samples for the analysis of hydrocarbons and other chemical contaminants will be sent to the appropriate NRDA/Trustees contractor (Alpha Analytical Laboratories for hydrocarbons and metals, Columbia Analytical Services for dispersants). Tissue sub-samples preserved for histology will be sent to the NOAA NWFSC Montlake Facility for histological preservation. A sub-set of egg samples will be sent to NOAA Alaska Fisheries Science Center in Kodiak, AK.

Water samples and samples for chemical analysis and egg samples for egg morphometry analysis will need to be offloaded from the vessel using runner boats and shipped to Alpha Analytical Laboratory and NOAA AKFSC, respectively every two days. The remaining samples can be stored on board the vessel and transferred during the port call or at the end of the cruise.

Each laboratory shall simultaneously deliver raw data, including all necessary metadata, generated as part of this work plan as a Laboratory Analytical Data Package (LADP) to the Trustee Data Management Team (DMT), at which point they will be uploaded to NOAANRDA.org and available to the Trustees and BP (or Cardno ENTRIX on behalf of BP). The electronic data deliverable (EDD) spreadsheet with pre-validated analytical results, which is a component of the complete LADP, will also be delivered to the secure FTP drop box maintained by the Trustees' Data Management Team (DMT). Thereafter, the DMT will validate and perform quality assurance/quality control (QA/QC) procedures on the LADP consistent with the authorized Analytical Quality Assurance Plan, after which time the validated/QA/QC'd data shall be made available simultaneously to all Trustees and BP (or Cardno ENTRIX on behalf of BP). Any questions raised on the validated/QA/QC results shall be handled per the procedures in the Analytical Quality Assurance Plan and the issue and results shall be distributed to all parties. In the interest of maintaining one consistent data set for use by all parties, only the validated/QA/QC'd data set released by the DMT shall be considered the consensus data set. In order to assure reliability of the consensus data and full review by the parties, no party shall publish consensus data until seven days after such data has been made available to the parties. Also, the LADP shall not be released by the DMT, LOSCO, BP or Cardno ENTRIX prior to validation/QA/QC absent a showing of critical operational need. Should any party show a critical operational need for data prior to validation/QA/QC, any released data will be clearly marked "preliminary/unvalidated" and will be made available equally to all trustees and to BP (or Cardno ENTRIX on behalf of BP).

All materials associated with the collection or analysis of samples under these protocols or pursuant to any approved work plan, including any remains of samples and including remains of extracts created during or remaining after analytical testing, must be preserved and disposed of in accordance with the preservation and disposal requirements set forth in Pretrial Orders ("PTOs") # 1, # 30, #35, # 37, #39 and #43 and any other applicable Court Orders governing tangible items that are or may be issued in MDL No. 2179 IN RE: Oil Spill by the Oil Rig "DEEPWATER HORIZON" (E.D. LA 2010). Destructive analytical testing of oil, dispersant or sediment samples may only be conducted in accordance with PTO # 37, paragraph 11, and PTO # 39, paragraph 11 and any other applicable Court Orders governing destructive analytical testing that may be issued in MDL No. 2179 IN RE: Oil Spill by the Oil Rig "DEEPWATER HORIZON" (E.D. LA 2010). Circumstances and procedures governing preservation and disposal of sample

materials by the Trustees must be set forth in a written protocol that is approved by the state or Federal agency whose employees or contractors are in possession or control of such materials and must comply with the provisions of PTOs # 1, # 30, # 35, 37, #39 and #43.

7.0 References:

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References to the studies cited in this work plan are for background and context only. Approval of this work plan does not constitute endorsement of, or agreement with, the methods, analysis, or conclusions of any study cited herein.

8.0 Costs:

The Parties acknowledge that this budget is an estimate, and that actual costs may prove to be higher. BP's commitment to fund the costs of this work includes any additional reasonable costs within the scope of this approved work plan that may arise. The Trustees will make a good faith effort to notify BP in advance of any such increased costs.

The project costs indicated below, including Vessel Costs for the NOAA R/V *Pisces* below are to be submitted by Trustees for reimbursement by BP.

Budget Section #1. Research and Vessel Costs to be submitted by Trustees for reimbursement by BP.

Pisces vessel costs (including mobilization and demobilization): $650,000

Personnel costs (including preparation and participation in cruise, and reporting): $325,000

Histology (NOAA Fisheries): $50,000

Reproductive health assessment: $60,000

Total: $1,085,000

Attachment 1: Crab embryology methods

Below is the protocol for characterizing the status and condition of crab eggs, including egg staging, egg morphometry, and fecundity. To prepare for sampling the crab eggs the abdominal flap (with eggs) should be removed by cutting the flap along the base of the shell making sure no eggs are lost. Eggs may then be removed for each method below:

Egg Staging
Randomly sample four egg clumps (with approximately 20 eggs each) from different locations around the clutch with forceps. Each egg clump should be removed and immersed in a separate Davidson's Fixative. Embryo developmental stages will be determined using a compound microscope with appropriate magnification to identify developmental stages (Moriyasu and Lanteigne 1998).

Egg morphometry
Randomly sample four egg clumps (with approximately 20 eggs each) from different locations around the clutch with forceps. Each egg clump should be removed immersed in a separate vial of seawater with 1/3 eggs, 1/3 seawater, 1/3 headspace. Vials must be kept chilled and seawater replaced every two days while at sea and examined under a dissecting microscope immediately upon arrival to the laboratory. If the samples do not get analyzed prior to egg degradation egg morphometry measurements will not be possible.

Back in the laboratory, digital images of fresh eggs from each female will be taken with a digital camera attached to a compound microscope at appropriate magnification. Using image analysis software, egg area and maximum, minimum and average diameter will be measured. Once embryos are discernable, embryo area and yolk area will also be measured and percent yolk calculated. Lastly, for eyed embryos, eyespot area and maximum, minimum and average diameter will be measured.

Fecundity
After the eggs are extracted for egg staging and egg morphometry a portion of the clutch (abdominal flap and eggs) should be frozen at -20 degrees C.

Eggs will be enumerated at NOAA AKFSC on the portion of the egg mass provided using dry weight methods. Embryos will be carefully stripped off of the pleopods and then two random samples of 250 embryos will be counted. The subsamples and remaining embryos will be dried at 60°C until a constant weight is achieved. Eggs will be enumerated by dividing the total dry weight of embryos by the average of the two estimates of individual embryo dry weight obtained from the subsamples. Fecundity will be determined by summing the numbers of eggs calculated in the subsample provided to NOAA AKFSC and the sub-sample provided to Alpha Analytical Laboratories for chemical analyses.